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NUTRITION REVIEWS

VOL. 9 MARCH, 1951 No. 3

RESEARCH AND DEVELOPMENT IN THE FOOD PROCESSING INDUSTRY*

There is no need of discussing with you the importance of research to our over-all economy, standard of living, or world problems. We all know that research is basic to progress and may be basic to our survival. However, using my own company as an example, I would like to try to bring you up to date a little on what is going on in research throughout the food processing industries.

Our company is really a merger of many smaller companies, each with its own products. The initial thought back of the merger was to promote efficiency and economy through increased volume, improved technics in various fields of operation, and better utilization of manpower.

There was one thing we learned early: the average specialty product—brand name product—has about the same life span as an individual. It is born, it has its vigorous youth, it reaches maturity; then old age sets in, and it tends to sink back. Another way of putting it is to say that over a period of time, a successful specialty becomes a commodity. As such it becomes subject to the laws of a commodity, with increasingly lower gross spread and a resultant lower net profit.

So if you just stand still with an unchanging group of products, your best endeavors to keep them modern with new faces and new uses cannot prevent retrogression. You must always have research on every phase of your operations—from the standpoints of processing, quality, uses, and packaging—just to protect your current business. But for real progress you must go beyond this into fields of new endeavor.

To me this is where the research end of our business becomes the prime factor in its growth or progress. It is absolutely essential to develop new products. And development of new products is a research job.

For this reason, despite war and depression we have never reduced our research budget. Research has been basic in our plans to such a degree that we are now spending two and a half million dollars a year on research. Research has been one department of our business which has shown a steady increase. In addition, we have associated ourselves with many other research endeavors outside the field of our own immediate company interests.

It was research, for example, that led to the remarkable developments in frozen foods. It was through research that Clarence Birdseye showed the advantage of quick freezing under pressure, a process which resulted in the formation of small ice crystals and less cell perforation than was previously possible in a slow-freezing process. But that was only where research began.

In the early days of frozen foods there was no adequate refrigeration either in the stores, to keep the foods in proper frozen condition, or in trains, to protect them in transit. Neither were there commercial warehouses which could store them properly. Twenty-five years ago the whole idea of frozen foods for consumers was a dream. It has cost us several million dollars to develop this quick-freezing process and to get frozen food products into national distribution We were accused of extravagance, but today—thanks largely to research—there is a major industry where none existed before.

This is one story. There are countless others.

Take our gelatin product—Jell-O. In the beginning we put the flavors in, but often they were gone before the package reached the consumer. To capture those flavors and

* Excerpts from a talk before the Industrial College of the Armed Forces, Washington, D. C., September 21, 1950.

keep them was a very difficult research job, but research was up to the task and may be credited with saving many thousands of dollars which might have been dissipated in waste and consumer dissatisfaction.

Precooked rice is another research development.

There is an interesting story in research contributions to the decaffeinization of coffee. When we first used that process, it took eighty hours to complete the decaffeinization. Today it takes eight hours. Does research pay? We know that it does.

What is going to happen next? One of the tasks of research is to answer this question. Whole new areas are bound to open up. The next step may be in electronics. We may be headed toward some new kind of food sterilization or preservation. Radioactive materials have opened up an entirely new field. Nobody knows yet just where they are going to take us. To stay out in front, a company must be willing to send challenges into the unknown—to make the best use of every kind of research. I am sure that, because of the importance of foods to the armed forces, you are vitally interested in the answers to these questions.

From the standpoint of industry generally, scientists are going ahead today with research projects which have a direct connection with your food problems. They are working on the development of antioxidants as a means of protecting fats and oils and providing long-shelf life. They are developing emulsifiers to improve the quality and the performance of lard and other products. There are the vitamin fortifications in many forms. You may have read about the new hormone which causes sheep to produce lambs twice a year instead of once. These research projects are only a few of those which can have a very definite effect on our future—both military and civilian.

Alongside direct research into food problems, there is another field of research vitally important to the food industry and the consumer—this is the field of packaging. Packaging doesn't mean just a piece of paper to wrap the product in for delivery. We must know what will happen to the product under various conditions.

An example—for years we had been wasting literally thousands and thousands of dollars in the way we packaged our corn flakes. When they got to the homes, they would be just a little soggy. They had lost their factory crispness. Naturally, this meant loss of sales for us and waste for the consumer. At long last—too long—we got busy on packaging. Now the corn flakes are crisp when they come out of the oven, and they are crisp when they reach the home.

We cannot afford to overlook the value of research in this field of packaging, for without the most complete package protection for our products, and without the most appealing package saleswise, the value of our research into food processing itself may be considerably diminished or lost entirely. And every packaging problem is a more complicated one when military requirements are considered.

Do not overlook the importance of careful selection of research projects. I have here a record which shows that four thousand, five hundred current topics are being examined in the food field by six hundred and fifty research laboratories. As a company we must make our own selection of research projects with care and foresight. Then we must continually re-evaluate and review progress to prevent research from running along indefinitely with no hope of fruition. This whole important field of research could gain in efficiency through greater use of such procedures.

There are other fronts. Some time ago one of my associates said: "I wonder if this industry shouldn't broaden its viewpoint. We are getting our money from the public. I wonder if there isn't something that we should do for the public in a perfectly unselfish way." As a result of that conversation, The Nutrition Foundation was formed. Dr. Karl Compton, head of the Massachu-

setts Institute of Technology, took the chairmanship. He enlisted Dr. Charles Glen King from the University of Pittsburgh as scientific director.

This organization today has drawn into action a group of eminent scientists from the universities and professional institutions. It has an industry committee of outstanding men. They are tackling very definite problems of a basic nature, all of which have actual application. Their organization is a tool of information and practical value which could be put to tackling your problems.

There is also the group with which many of you are familiar—The Associates, Food & Container Institute. This group was established in 1947 for the purpose of developing more effective working relationships between the Quartermaster Food and Container Institute and the food and container and other related industries. Those who were responsible for the formation of The Associates were convinced that, in the event of another war, we could not afford to be caught in an acute state of unpreparedness as we were in both previous world wars. Today there are two hundred corporations represented in The Associates, each with a representative of its research staff working with the Quartermaster group to promote dissemination of scientific information pertaining to foods and containers, to function as a clearing house on research activities and eliminate unnecessary duplication, to evolve and maintain a research mobilization plan, to determine food and container research problems and encourage scientists to pursue them, to keep members of The Associates informed of the Institute's activities, and to assist the Institute with technical information relating to industrial experience and practice.

There isn't any question that The Associates have helped the Quartermaster group do a better job in realizing Secretary Pace's objective that the boys in the front line may be fed as well as in any other place and better if we can make it that way.

However, an even greater potential contribution can be made by The Associates to the Quartermaster research for the good of the country. Perhaps you in your contacts with it may be able to help us develop this potential. We still have a long way to go.

I doubt whether there is a person here who does not feel the overwhelming importance research has to the success of our domestic and military operations. It is of such importance that we can no longer afford to keep research in an ivory tower laboratory. Research *is* a technic, but it is also a state of mind, and it is a state of mind that each of you here today with your great responsibilities must cultivate in yourselves and those with whom you work. It is a state of mind which questions, which challenges, which refuses to accept the pattern of things as they are. It is a state of mind which looks for and uses every resource at its command. It is a state of mind which finds the best method and is not satisfied until a better one has been found.

Dr. James B. Conant, President of Harvard University, has said that to get the best results in fundamental research work "You should get the best men obtainable, finance them liberally and leave them severely alone." To get the best results in applied industrial research, he recommends "Getting the best men obtainable, financing them liberally and then *keeping their eyes everlastingly on the target.*"

I'm afraid we have today a target or a goal of such enormity that it is difficult not to stare at it in a fascinated paralysis, but it is more easily seen than reached. If we have a long way to go, it will be largely your leadership, your insistence on the quickest route, which will enable the country and the world to reach it.

CLARENCE FRANCIS, *Chairman*
General Foods Corporation

METHODOLOGY OF DIETARY SURVEYS

Before World War II, E. J. Bigwood ("Guiding Principles for Studies on the Nutrition of Populations," *Technical Commission on Nutrition, Health Organization, League of Nations, Geneva (1939)*) had prepared for the League of Nations a concise manual of practical nutrition which can still be considered a model of exposition. The manual contained a section on the methodology of dietary surveys.

Since the end of the war, active interest in dietary surveys has been widespread throughout the world, and several comprehensive national studies have been published reviewing, more or less critically, methods and achievements in this field. A British study by the Commonwealth Bureau of Animal Nutrition ("Technique and Interpretation of Dietary Surveys," *Rowett Research Institute, Bucksburn, Aberdeenshire (1948)*), an American study ("Dietary Surveys and Their Interpretation," *U. S. Department of Agriculture, Bureau of Human Nutrition and Home Economics, Washington (1948)*), and a French study (J. Tremolieres and I. Pequignot, *Bull. Inst. nat. d'hyg.* **3,** *225 (1948)*) may be quoted as examples.

The Nutrition Division of the Food and Agriculture Organization has distributed a manual prepared by one of its members, Thelma J. Norris ("Dietary Surveys: Their Technique and Interpretation," *FAO Nutritional Studies, No. 4, Dec. 1949*), based on worldwide experience in dietary surveys, which on the whole represents a considerable advance over previous publications on the subject.

The manual is comprised of five sections. The first section deals with the purposes of dietary surveys. This section might have been somewhat longer and more explicit, for the benefit of those readers who are not primarily nutritionists, for example, health officials, agricultural planners, etc. For the professional nutritionists toward whom the manual is primarily directed, it is no doubt adequate.

The second section deals with such general considerations as planning of surveys, including selection and training of personnel, choice of samples, use of food composition tables, etc.

The third section describes the various methods used in dietary surveys in what is perhaps the best such description written to this date. This reviewer feels, however, that a somewhat more critical comparison of the relative merits of the methods described would have been helpful.

The fourth section deals with what is perhaps the most difficult aspect of this subject, the treatment of the dietary survey data. There again the factual presentation is excellent, but one may wish that some of the pitfalls and difficulties encountered in past surveys had been more critically discussed.

Finally, a fifth section deals with "Dietary Surveys in Underdeveloped Areas." Miss Norris has had personal experience with dietary surveys in connection with the New Guinea Surveys and has benefited from the knowledge of other members of the Nutrition Division of FAO who have conducted dietary surveys in South America, Africa, Asia, and the Pacific Islands. This section thus covers authoritatively an entirely new subject and should prove useful particularly to workers starting nutrition work in areas where none has been done. Useful appendices on food classification, logbooks, inventories, and schedules for food lists, are included.

It may be safely concluded that this little manual should find its place in the library of all public health nutritionists.

COBRA VENOM AND THROMBOPLASTIN

An interesting by-product of the work of H. Dam and his collaborators, at the Polytechnic Institute of Copenhagen, on plasma coagulation (*see Nutrition Reviews* **9,** *36 (1951)*) concerns the mechanism of the anticoagulating property of cobra venom. It had been known for a long time that this secretion contains lecithinase A, an enzyme which splits off unsaturated fatty acids from phospholipids, thus forming lysolecithins. These substances, in turn, are responsible for the hemolytic action of cobra venom.

Similarly, it had been generally assumed that the anticoagulant effect of the venom was due to the inactivation or destruction of the phospholipid component of thromboplastin by the lecithinase. The fact that this explanation was contrary to the old observation of A. Calmette ("Les Venins, les animaux venimeux et la sérothérapie antivenimeuse," *Paris (1907)*) that the anticoagulant component is destroyed if the venom is heated for one hour at 70°C., while the general toxicity and the lecithinase activity resist this treatment, had been largely disregarded. The whole question has now been considered by H. Dam and one of his collaborators (I. Kruse and Dam, *Biochim. et biophys. acta* **5,** *268 (1950)*).

The material used was chick blood and plasma. Cobra venom was reconstituted from the desiccated secretion. The basic method was a study of clotting times in various circumstances. For example, to the citrated plasma were added calcium salt and preparations of thromboplastin incubated during increasing lengths of time with cobra venom; the correspondingly increasing clotting times were determined. In another series, chicken plasma was incubated directly with cobra venom for various lengths of time and preparations of thromboplastin added later. It was found that the latter procedure prolonged clotting time to a much lesser extent than incubation of thromboplastin with cobra venom. On the other hand, cobra venom had no effect if plastin, rather than thromboplastin, was used.

These results made it plain that inactivation of thromboplastin was the cause of the anticoagulant effect of cobra venom. The authors demonstrated that the anticoagulant property was independent of the lecithinase activity by confirming that this property disappeared on heating while lecithinase activity did not. Furthermore, the activity of the thermolabile anticoagulant concentrate which they prepared by salt fractionation did not parallel the concentration of the lecithinase activity.

Finally, the action of venom antiserum was studied. It was shown that it consisted primarily in the protection of thromboplastin, as, in order to be effective, addition of antiserum has to take place before the addition of cobra venom. If the antiserum were added to the already incubated mixture of plasma and venom, very little protection was afforded. These observations on the action of antiserum thus give additional proof of the postulated action of the venom.

Besides its immediate practical interest as a study of a classic toxicologic problem, this work adds to the knowledge on an important physiologic question. Cobra venom can now be added to the other tools—vitamin K deficiency, Dicumarol poisoning, etc.—which Dam and his school have used so skillfully to further knowledge of the mechanism of blood coagulation.

CONVERSION OF THIAMINE INTO ACTIVE COENZYME

One of the significant aspects of the biochemistry of vitamins, the transformation to active coenzymes, has been furthered by the report of G. Smits and E. Florijn (*Biochim. et biophys. acta* **5,** *535 (1950)*).

The initial observation which prompted

the study was that no conversion of thiamine to thiamine pyrophosphate, the coenzyme which is the physiologically active form of this vitamin, occurred in human or rat blood to which oxalate had been added as an anticoagulant, while the conversion did occur in heparinized or defibrinated blood. Failure of this conversion did not appear to be due to removal of the calcium ion, since the reaction did proceed in citrated blood. Since oxalate is a known inhibitor of glycolysis, the authors proceeded with a systematic study of other glycolysis inhibitors, on the supposition that the phosphorylation of thiamine may be coupled with the energy-yielding reactions of glycolysis. The rate of synthesis of thiamine pyrophosphate by both rat erythrocytes and leucocytes was measured.

In the presence of thiamine, leucocytes have a much greater capacity to synthesize the coenzyme than erythrocytes; they contain some one hundred and sixty times as much thiamine pyrophosphate as red cells. Coenzyme synthesis by leucocytes is inhibited more by cyanide than by oxalate, while the reverse is true of the synthesis by erythrocytes. The latter findings may be correlated with the known facts that red cells acquire their energy by glycolysis, while white cells acquire energy mostly by respiration.

To further this thesis, Smits and Florijn calculated the relative number of high-energy phosphate bonds, stored as adenosinetriphosphate (ATP), synthesized by rat leucocytes by respiration, and erythrocytes by glycolysis. They also calculated the effect of various inhibitors on the rate of coenzyme synthesis. These calculations showed that leucocytes produce much more ATP than erythrocytes, that ATP production by leucocytes is much more inhibited by cyanide than by oxalate, and finally that ATP synthesis by erythrocytes is more inhibited by oxalate than cyanide. The parallelism noted between thiamine pyrophosphate and ATP synthesis led the investigators to the conclusion that energy is needed for synthesis of thiamine pyrophosphate from thiamine, that the energy may be obtained either from glycolysis or respiration, and that the effect of the various inhibitors used was both qualitatively and quantitatively that to be expected.

Further work demonstrated that the lower the initial concentration of thiamine, the greater was the percentage of thiamine converted to the coenzyme, but even at the lowest concentration a complete conversion was not obtained. In studying the kinetics of the conversion, a progressive decrease in the reaction rate was observed. Since the free coenzyme does not exist in the cell, being either rapidly destroyed by phosphatases or combined with protein, the authors first postulated that there might be a lack of the carrier protein. However, by addition of thiamine after increasing periods of incubation, the investigators eliminated this hypothesis. Maintenance of an adequate level of glucose during incubation decreased the apparent damage to the synthesizing mechanism, but glucose concentration was not the entire reason for the decreasing reaction rate of the synthesis.

Mutual relations in metabolism are of interest and well illustrated by the work reviewed. Thiamine in its coenzyme form is essential in the liberation of energy from the glucose molecule, and this energy is in turn necessary to produce the coenzyme in tissues from the thiamine obtained in the diet.

DIETARY TREATMENT OF HYPERTENSION. I. CLINICAL STUDIES

Reduction in blood pressure in hypertension by use of diets restricted in salt and other nutrients has had clearcut success in the hands of some workers and has aroused great interest in the relation between nutrition and this disease, one of the most important causes of morbidity and mortality in this country. Since publication of a previ-

ous review (*Nutrition Reviews* **6,** *293 (1948)*) further clinical studies have shown the effectiveness of the rice diet and certain low sodium diets in the treatment of at least some patients with hypertensive disease.

A note of doubt was cast on the effectiveness of rigid sodium restriction by H. Chasis *et al.* (*J. Am. Med. Assn.* **142,** *711 (1950)*) who found significant reduction in systolic and diastolic blood pressure in only 4 of 12 patients studied. The authors believed the positive results to be of doubtful significance since the fall in blood pressure often occurred before or immediately after institution of the rice diet. Thus, the change was not correlated specifically with the alteration in the diet. It may be noted, however, that the urinary chloride excretion in these patients, while they were maintained on the rice diet, was considerably higher in many cases than that observed by W. Kempner (*Ann. Int. Med.* **31,** *821 (1949)*). The patients had been maintained on a ward diet until it was thought that a stable blood pressure had been reached. The rice diet described by Kempner (*Nutrition Reviews* **6,** *293 (1948)*) was then instituted for from fourteen to ninety-eight days. Large quantities of sodium chloride (30 g.) were added to the diet for a few days at the end of the rice diet period in 5 patients. The ward diet was reinstituted in 4 patients.

Similar doubt as to the clinical effectiveness of the rice diet is supplied by the data of R. E. Weston *et al.* (*J. Clin. Invest.* **29,** *639 (1950)*), whose patients were also followed for three or more weeks on a ward diet, following which the rice diet was instituted. During administration of the rice diet a pronounced drop in systolic and diastolic blood pressures occurred in only one patient, and a decrease in systolic blood pressure only in a second patient. Presumably significant alterations in the blood pressure were not observed in the other 8 patients. The twenty-four-hour chloride excretion is not presented in this report.

Further studies have been made by V. P. Dole *et al.* (*J. Clin. Invest.* **29,** *1189 (1950)*) on 6 patients with hypertensive cardiovascular disease. Again the patients were maintained on an unrestricted hospital diet for from fourteen to thirty days of control. Thereupon the rice diet was instituted, modified by increasing or decreasing the quantity of rice or the quantity of fruit and sugar allowed daily. Unequivocal reduction of blood pressure occurred in 5 of the 6 patients while on the rice diet. In one patient the drop was so marked that at the end of the second period 10 g. of sodium chloride were administered, accompanied by a marked rise in blood pressure back to the previous level. Subsequent omission of the sodium chloride in this patient again resulted in prompt reduction of blood pressure. No significant change in blood pressure on the addition of salt to the rice diet was observed in another patient, and in a third an actual decline in blood pressure occurred.

The reduction in blood pressure observed on the rice diet was of rather gradual occurrence in most instances. In addition to decreasing hypertension, decrease in heart size and change in optic fundi and electrocardiograms toward normal also occurred. All patients in this series lost weight during the initial period on the rice diet. Later the weights of most of the patients tended to level off although weight gain occurred in some.

An extensive investigation of the effects of the rice diet in hypertension has been presented by D. M. Watkin, H. F. Froeb, F. T. Hatch, and A. B. Gutman (*Am. J. Med.* **9,** *428, 441 (1950)*). The effect of prolonged hospitalization (three to twenty-one weeks, average nine and three-tenths weeks) on a regular hospital diet was studied in 86 patients as a preliminary observation. This period served as a control for the study of 50 of these patients during a subsequent period on the unmodified Kempner rice diet.

Bed rest and the hospital regimen did lead to certain changes although they were highly variable from patient to patient. Definite symptomatic improvement occurred in many of these patients. When present, the follow-

ing symptoms improved in the order named: edema, dyspnea, angina pectoris, nausea and vomiting, dizziness, orthopnea, severe headache, and disorientation. Great care was taken to assure standardization of the blood pressure measurements. In a number of patients there was a striking drop. Nevertheless, comparing the mean basal blood pressure during the first week of hospitalization with that in the final three weeks, the drop in most of the patients was small and, in fact, some patients had a distinct and sometimes marked rise in blood pressure. The average for the 86 patients was a drop of 8 mm. of mercury systolic and 3 mm. diastolic. Reduction in heart size during this period of hospitalization occurred in only a few of the patients. Likewise, in most subjects there was no change in the electrocardiogram.

Of the 33 patients with class 3 or 4 severity of hypertensive retinopathy, only 12 improved while 6 got worse. There was no significant change in the serum cholesterol and phospholipid concentrations. In summary, the data suggest, and the authors point out, that the response of patients with essential hypertension to simple hospitalization is so variable that prolonged observation is necessary to appraise the effects of other and more specific therapy. Thus, the careful study in these patients and the prolonged hospitalization were justified.

The series of 50 patients studied on the rice regimen were taken from the group of 86 patients who had gone through the control weeks of hospitalization successfully but still had hypertension. Great care was taken to stabilize the entire regimen, including physical and mental activity. The rice diet as described by Kempner was adhered to strictly and provided between 1800 and 2400 calories daily, furnishing about 29 g. of protein, between 420 and 570 g. of carbohydrate, and less than 3 g. of fat. The daily dietary sodium was between 2.5 and 7.0 mEq., while 73.5 mEq. of potassium, 13.0 mEq. of calcium, and 15.1 mEq. of phosphorus were given daily. Fluids were limited to 1 liter. The diet contained essentially no cholesterol.

A multivitamin capsule containing thiamine, riboflavin, nicotinamide, pyridoxine, calcium pantothenate, vitamins A, C, D, and E, and 0.2 g. of ferrous sulfate were given daily. The diet contained more protein than stated by Kempner, presumably because of the kind of rice used, namely a Texas-grown, long-grain, American Patna. This rice contained 1.45 per cent of nitrogen by Kjeldahl analysis as compared with 1.42 per cent for brown rice, 1.22 per cent for long-grain rice, between 1.03 and 1.20 per cent for glutinous rice, and between 0.85 and 0.95 per cent for pearl rice. Difficulties were of course encountered in obtaining the cooperation of the patients. To quote the authors: "Many of our patients began the rice diet and more continued it with considerable reluctance. It was necessary to bolster morale by explaining the purpose of the regimen and by reporting encouraging details of improvement as they developed."

Contamination of the diet with salt, whether by design or not, was controlled by daily determination of urine chloride. During the time of marked depletion of the New York City reservoirs, an increase in sodium chloride content of water which was used for steaming rice and dishwashing occurred and appeared as an increase in urinary chloride.

Improvement in symptomatology occurred in a far higher number and percentage of patients on the rice diet than during the control period. Thus, edema disappeared in all patients in whom the symptom occurred, severe headaches disappeared in 64 per cent as compared with 19 per cent of the control, dyspnea in 62 per cent compared with 35 per cent, and orthopnea in 43 per cent compared with 36 per cent. Both systolic and diastolic blood pressures fell in most of the patients studied. In fact, 31 of the 50 had a drop of greater than 10 mm. of mercury in both pressures, and 13 patients sustained a drop of more than 20 mm.

Seven patients finished the rice diet period with pressures below 140/90. The pressure failed to fall or rose slightly in a few patients. The average drop in blood pressure in the ten and a half weeks of study was 29 mm. of mercury systolic and 16 mm. diastolic compared with 9 and 3 mm., respectively, during the ten and one-tenth weeks of the control period for these 50 patients. With regard to hypertensive retinopathy, 86 per cent of the patients with class 4 retinopathy improved on the rice diet as compared with only 29 per cent during the control period. A similar distribution was seen in those with grade 2 or 3 severity of retinopathy. Unquestioned favorable electrocardiographic changes occurred in some of the patients but did not correlate well with other indications of improvement so that the authors were cautious in interpreting them as evidence of significant improvement.

Of the 33 patients in whom heart size was determined, both in the control period and during the rice diet administration, 19 patients had a reduction in transverse diameter of greater than 1 cm. during the rice diet period compared with only 2 patients with a similar reduction in size during the control period. Most of the patients lost weight, associated with diuresis, during the first few weeks of the rice diet. Thereafter weight gradually stabilized and then remained constant or increased to previous levels, but this time presumably not due to retention of water but rather to true tissue gain. A frequency distribution chart of the total blood cholesterol content demonstrated a significant reduction in a large number of patients, particularly those with initially high blood concentrations. The total cholesterol fell an average of 40 mg. per cent representing a fall of only 5 mg. per cent free cholesterol and 35 mg. per cent in the ester fraction, indicating that the greatest reduction occurred in the latter fraction.

Addition of sodium chloride to the diet by means of altering a placebo capsule of sucrose to that of sodium chloride, resulted in elevation of blood pressure in most patients so that the authors concluded that the most significant factor in the reduction of the elevated blood pressure and alteration in clinical status was the extremely low sodium intake on the rice diet. They point out the difficulty in sustaining this treatment because the sodium intake must be maintained at such low levels that little other than the rice diet can be used. Such a diet imposes great hardship on the patient, making it "virtually impracticable for general use." They also point out that for future study the upper limit of sodium toleration must be determined and also what other foods can be allowed in the diet and still be within the intake of sodium required.

In summary, of the 75 patients studied in the papers covered by this review, approximately one-third had a clearcut reduction in blood pressure attributable to the rice diet, most importantly to its low sodium content. Although this dietary therapy is not a panacea for the treatment of all patients and all types of hypertensive disease, it is unquestionably an effective measure in many patients. Further investigations must attempt to make the diet more palatable and liberal so that it can be more widely applied. The mechanism of the effectiveness of the diet must be studied. Preliminary observations on the metabolic alterations induced in the body by changing to the rice diet are presented in Part II which follows.

DIETARY TREATMENT OF HYPERTENSION. II. METABOLIC AND RENAL STUDIES

The study of hypertension, presents many aspects, one of the most interesting and important of which in recent years has been that of renal hemodynamics, which are clear-

ly altered in this disease. It is not surprising, therefore, to find studies of the effect of specific dietary regimens upon not only blood pressure but also renal hemodynamics in patients with hypertensive disease. Two such studies have been published (H. Chasis *et al.*, *J. Am. Med. Assn.* **142,** *711 (1950)*; R. E. Weston *et al.*, *J. Clin. Invest.* **29,** *639 (1950)*). Before reviewing these two papers it will be necessary to examine briefly the alterations in renal hemodynamics which are regularly found in patients with hypertensive disease.

Normally, as arterial blood is supplied to the kidneys it is presented first to the glomerulus, through which there is passage of a large volume of plasma ultrafiltrate containing crystalloids in approximately the same concentration as they occur in the plasma delivered to the kidney. The glomerular filtrate then passes down the kidney tubules which are supplied by the blood that leaves the glomerulus, where some substances in the filtrate, for example, water and salt, are reabsorbed and other substances are excreted by the tubular cells. Appropriate methods are now available for measuring the rate of blood flow (plasma flow) through the kidney, the quantity of glomerular filtrate produced per unit of time, the capacity of the kidney to excrete certain substances, and the so-called "filtration fraction." The filtration fraction is that fraction of plasma flowing through the kidney at any one time, which is filtered through the glomerulus.

W. Goldring and H. Chasis ("Hypertension and Hypertensive Disease," *The Commonwealth Fund, New York (1944)*) in reviewing the various aspects of hypertension, note that progressive changes in renal function are characteristic of hypertensive disease in man. The earliest constant changes occurring in well-established hypertension are three in number: reduction in maximal tubular excretory capacity, diminution in the rate of renal plasma flow, and increase in the filtration fraction. These changes occur before abnormalities in the usual clinical kidney function tests are evident. The rate of glomerular filtration diminishes gradually in patients with hypertension but begins considerably later in the disease than the derangement in the other functions mentioned above.

The study by Chasis *et al.* concerns 12 patients with essential hypertension who were maintained during a pretreatment period on a ward diet which provided approximately 2500 calories a day divided between 250 and 300 g. of carbohydrate, 70 g. of protein, and 85 g. of fat. There were between 6 and 7 g. of sodium chloride in this diet which was continued for from fourteen to seventy-nine days, that is, until it was felt that the blood pressure had become stabilized. Actually it was difficult to establish a stable blood pressure, as indicated by the difficulty in interpreting subsequent falls in 2 patients while maintained on the rice diet. At any rate, as soon as the blood pressure was thought to be stabilized, the rice diet described by W. Kempner (*Nutrition Reviews* **6,** *293 (1948)*) was provided for from fourteen to ninety-eight days. Large quantities of sodium chloride (30 g.) were added to the diet for a few days at the end of the rice diet period in 5 patients. The ward diet was reinstituted in 4 patients.

Nine of the 10 patients studied had a significant reduction in the rate of glomerular filtration during the rice diet. In the 5 patients subsequently given sodium chloride, the filtration rate increased to approximately the control value. Likewise, there was a decrease in the renal plasma flow in 8 of the 10 patients consuming the rice diet. However, in 6 of the patients the decrease in renal plasma flow was less, proportionately, than that of the glomerular filtration rate so that the filtration fraction was significantly decreased. There was no change in the filtration fraction in the other 4 patients. Finally, 6 of the 10 patients had a definite decrease in the maximal tubular excretory

capacity during administration of the rice diet. Reinstitution of the ward diet led to a return to the control values.

The second study, by Weston *et al.*, reports the result in 9 hypertensive patients who were also followed for three or more weeks on a ward diet described as containing 65 g. of protein and approximately 3 to 4 g. of sodium. The change was then made to the rice diet described by Kempner (*loc. cit.*). The period on the Kempner diet was three weeks or, in several patients, for considerably longer periods. In addition to the low sodium diet, mercurial diuretics were given to 3 of the patients while they were maintained on the rice diet. This was done to increase the loss of sodium from the body.

In addition to the renal function studies, plasma volumes were measured by the Evans-blue dye method. In contrast to the first study noted above, no conclusive changes in renal plasma flow or maximum tubular excretory capacity on the rice diet alone were noted. There was a decrease in glomerular filtration rate, however, in 5 of the 6 patients. When mercurial diuretics were administered to the patients on the rice diet, a decrease in renal plasma flow and reduction in filtration fraction did occur. A fall in plasma volume was noted in 5 of the 9 patients. Infusions of concentrated sodium chloride were given to some patients at the end of the experimental period. This procedure induced a rapid increase in the filtration rate. The authors point out that these changes in renal function, particularly the decreased glomerular filtration rate, occurring in patients maintained on the rice diet, are of potential danger. The possibility of either severe azotemia or a marked or irreparable loss of electrolytes must be considered and carefully watched for in caring for these patients, especially and perhaps only when mercurial diuretics are used.

R. J. F. Murphy (*J. Clin. Invest.* **29,** *912* (*1950*)) has contributed another study on the plasma volume and the extracellular fluid space in hypertensive patients subjected to prolonged maintenance on the rice diet, although he did not undertake investigation of renal function. In all, 17 patients were studied. The plasma volume was measured by the Evans-blue dye method and the extracellular fluid volume by dilution of thiocyanate. Here again there was a distinct fall in the plasma volume in all but 2 of the patients and a drop in the interstitial fluid (total extracellular fluid minus plasma volume) in all the patients. There was no change in the plasma protein concentration in these patients so that the total circulating protein dropped significantly. The authors emphasized that others who have found no change in plasma volume while maintaining patients on a low sodium diet (G. A. Perera and D. W. Blood, *Ibid.* **26,** *1109* (*1947*)) were using diets which contained between 250 and 300 mg. of sodium daily. The diet used by Murphy was that described by Kempner and contained not more than 150 mg. of sodium. He suggests that there may be a critical level of sodium intake below 250 mg. in which these changes occur.

Careful and prolonged metabolic studies on 6 patients with hypertensive cardiovascular disease were made by V. P. Dole *et al.* (*J. Clin. Invest.* **29,** *1189* (*1950*)) while the patients were maintained on the Kempner rice diet. The patients were maintained in a metabolic ward during four periods of study. When this program had been continued for a month the diet was standardized so that exactly the same type of food was consumed each day for seventeen days while balance studies were conducted. A subsequent period, of twenty-eight days duration, differed only in that 2.5 g. of ammonium chloride were added to the diet. In a final, or fourth period, of twenty-seven days duration, 2.5 g. of sodium chloride were substituted for the ammonium chloride. Balance studies were likewise made. There were certain minor exceptions to these regimens, necessitated by the clinical states of the patients.

All the patients lost weight during the initial period on the rice diet. Later the weight of most of the patients tended to stabilize, although weight gain appeared in some. With regard to nitrogen balance, the 3 largest individuals failed to reach equilibrium during a first balance period; the other 3 patients were in balance. Thereafter nitrogen balance gradually appeared in all of the patients. The authors concluded that nitrogen balance can be obtained on the rice-fruit diet but the time required for adaptation is to be measured in months. Sodium and potassium equilibrium also occurred during the rice-fruit regimen. Considerably more metabolic data of interest were reported in this study as well as careful statistical analysis of the data obtained.

Such studies, complementary as they may seem to be to the clinical applicability of the rice diet, are essential to an appreciation of the accompanying metabolic effects and possible contraindications of the method, and they form the rational basis of future clinical development.

VITAMIN E IN THE NUTRITION OF CATTLE

In 1922 H. M. Evans and K. S. Bishop (*Science* **56,** *650 (1922)*) established that rats require vitamin E for successful reproduction, and since then the possible relation of this vitamin to reproductive failure in cattle has been investigated repeatedly. Although there have been many claims for success with vitamin E in the treatment of infertile conditions in dairy cattle (S. A. Asdell, *J. Dairy Sci.* **32,** *60 (1949)*), investigators at the University of Minnesota have reported results to the contrary. Nine heifers were raised successfully on a vitamin E-low ration and all calved normally (T. W. Gullickson, L. S. Palmer, W. L. Boyd, and F. C. Olson, *Ibid.* **27,** *634 (1944)*).

The Minnesota group later reported that sudden deaths, attributable to cardiac failure, frequently occurred when cattle were fed vitamin E-low rations throughout their life span (*Nutrition Reviews* **7,** *118 (1949)*). A subsequent report from the Minnesota laboratory is concerned with the influence of vitamin E on reproduction, health, milk production, and growth of cattle (T. W. Gullickson *et al.*, *J. Dairy Sci.* **32,** *495 (1949)*).

The rations used for these studies were composed of feedstuffs that were initially low in vitamin E, as determined by rat bioassay. The ration ingredients were either heat-treated or solvent-extracted to destroy or remove any vitamin E present.

Rice straw was fed as the roughage. A concentrate mixture, typical of those fed, had the following percentage composition: polished rice 25; brewers' dried grains 30; distillers' grains (solvent-extracted) 18; corn starch 11, dry skim milk 9; lard 4; bone meal 2; and iodized salt 1. Adequate amounts of vitamins A and D were included in the rations. The rations were designed to contain liberal amounts of all known essential nutritional factors except vitamin E.

Thirty animals were fed the vitamin E-low ration (8 males and 22 females). Included in the experiment were 13 of an original group (5 males and 8 females) and 17 that were their second, third, or fourth generation descendants. Four control animals, one male and 3 females, were fed the same ration supplemented with either alpha-tocopherol or tocopherol concentrates.

There was no indication that the vitamin E-low rations influenced the reproductive efficiency. The 6 bulls fed the vitamin E-low rations had an average breeding efficiency of 83.3 per cent. All sperm samples examined were found to be normal with respect to sperm activity and morphology.

Only thirty services were required to pro-

duce twenty-five pregnancies in 19 females during the experiment. All the first and second generation heifers from the original stock in the experiment conceived on the first service. These heifers had been raised on whole milk from cows receiving the vitamin E-low rations until they were 3 weeks old. They then received fresh skim milk until they were 6 months old. Rice straw was fed as roughage along with enough of the vitamin E-low concentrates to provide adequate protein and energy.

There were no abortions during the experiment and all calves were normal in size and vigor at birth. Fetal membranes were invariably expelled within a few hours after calving. No abnormalities were detected in the reproductive organs of the bulls or cows at autopsy.

Thirteen of the 28 animals that received the vitamin E-low rations for one year or longer died suddenly at ages ranging from 21 months to 5 years. Most of these animals experienced some loss of appetite for a month or more before death. It was emphasized, however, that the appetite loss, body weight loss, or any pathologic changes noted on postmortem examination were not sufficiently severe to indicate any specific cause of death. Slight brain, bowel, heart, and pancreatic hemorrhages were noted. The sudden deaths were considered as probably the result of cardiac injury, as previously reported by these workers.

There was no indication that vitamin E-deficient rations had any influence on growth rate. Two heifers (first generation) were less than 18 months when they calved and one bull mated successfully at 10 months of age, indicating that vitamin E deficiency likewise did not delay sexual maturity.

Neither milk nor butterfat production was adversely affected by feeding vitamin E-deficient rations. The Minnesota workers (T. W. Gullickson, J. B. Fitch, and L. O. Gilmore, *J. Dairy Sci.* **31,** *557 (1948)*) and others (F. Whiting, J. K. Loosli, V. N. Krukovsky, and K. L. Turk, *Ibid.* **32,** *133 (1949)*; P. H. Phillips, J. Kastelic, and E. B. Hart, *J. Nutrition* **36,** *695 (1948)*), had reported that feeding tocopherol to cows on normal rations had little or no influence on the quantity or fat content of the milk produced. On the other hand, P. L. Harris, W. J. Swanson, and K. C. D. Hickman (*Ibid.* **33,** *411 (1947)*) had reported earlier that vitamin E enhanced butterfat production of dairy cows.

The Minnesota workers point out that feeds commonly used in the rations for dairy cattle are relatively rich in vitamin E. Under practical conditions sudden deaths due to vitamin E deficiency would therefore be considered as extremely unlikely.

The experimentally induced vitamin E deficiency in cattle that often climaxes in sudden death is an interesting one. This terminal event is not, however, unique to this species since sudden deaths have also been reported to occur in rats fed vitamin E-deficient diets (E. L. Hove, D. H. Copeland, and W. D. Salmon, *J. Nutrition* **39,** *397 (1949)*). The sudden death in rats is believed to be caused by massive hemorrhages in the lungs and liver. The Minnesota workers noted some hemorrhages in their cattle although they were not considered severe enough to explain the sudden deaths.

The extensive studies of the Minnesota group establish rather conclusively that rations extremely low in vitamin E can be fed to cattle for extended periods without precipitating reproductive disturbances. It would seem unlikely, therefore, that a deficiency of this vitamin is involved in the reproductive failures that often occur in cattle under practical conditions. There are nevertheless extensive claims in the literature of the beneficial influence of vitamin E-rich supplements upon reproductive efficiency in cattle. Does this imply that natural feeds contain factors that are harmful to the reproductive system and that vitamin E

functions in counteracting these harmful effects?

The observation that cardiac failure is involved in vitamin E deficiency in cattle has tempted some clinicians to use this vitamin in the treatment of human cases of heart disease. Critical clinical studies have indicated, however, that vitamin E is of no value in the treatment of human heart ailments (*Nutrition Reviews* **7,** *118, 314 (1949)*).

BOUND FORMS OF PANTOTHENIC ACID

During the past decade a number of laboratories have reported evidence for the existence of bound pantothenic acid in tissues (*Nutrition Reviews* **7,** *348 (1949)*). The one for which a physiologic function has been demonstrated is coenzyme A, which was shown by F. Lipmann *et al.* (*J. Biol. Chem.* **167,** *869 (1947)*) to contain pantothenic acid, and it is now known to catalyze the acetylation of sulfanilamide, choline, acetate (to acetoacetate), and oxalacetate (to citrate).

G. D. Novelli, N. O. Kaplan, and F. Lipmann (*J. Biol. Chem.* **177,** *97 (1949)*) have contended that coenzyme A accounts for virtually all of the pantothenic acid which is present in animal tissues, but this conclusion has been challenged by T. E. King, L. M. Locher, and V. H. Cheldelin (*Arch. Biochem.* **17,** *483 (1948)*) who have partially purified another tissue conjugate of pantothenic acid which they call PAC and which is of larger molecular size than coenzyme A. It appears, furthermore, to contain more glutamic acid than coenzyme A although it is similar in its enzymatic requirements for liberation of free pantothenic acid (*Nutrition Reviews* **7,** *348 (1949)*).

PAC has no known coenzyme function although it is more active than free pantothenic acid in the stimulation of growth of *Acetobacter suboxydans* and certain other bacteria. Coenzyme A and certain enzymatic degradation products of coenzyme A which are inactive as coenzymes are also active in the *Acetobacter* assay (G. D. Novelli, R. M. Flynn, and F. Lipmann, *J. Biol. Chem.* **177,** *493 (1949)*) and thus total bound pantothenic acid determined by the *Acetobacter suboxydans* assay includes coenzyme A, fragments of coenzyme A liberated by a hydrolytic enzyme from avian liver, and PAC.

H. Nishi, T. E. King, and V. H. Cheldelin (*J. Nutrition* **41,** *279 (1950)*) have attempted to delineate PAC from coenzyme A in a series of experiments involving induction of pantothenic acid and glutamic acid deficiencies in rats, and comparison of the *Acetobacter* growth and acetylating activities of tissue extracts from deficient and litter-mate control rats before and after dialysis. In both normal and deficient tissues, free pantothenic acid was only a small fraction (3 to 15 per cent) of the total bound pantothenic acid (determined by *Acetobacter* assay) in agreement with the conclusions of Novelli, Kaplan, and Lipmann (*loc. cit.*). Free pantothenic acid, furthermore, tended to disappear more rapidly than bound pantothenic acid in rats restricted of the vitamin for sixty-one days. The percentage of the free vitamin retained varied from 53 per cent in liver to 10 per cent in brain, while retention of the bound vitamin (PAC or similar substances active in the *Acetobacter* assay) was 55 per cent of that of controls in liver and 75 per cent in brain.

In studies previously reported (*Nutrition Reviews* **7,** *348 (1949)*) in which coenzyme A activity was followed as an index of bound pantothenic acid in pantothenic acid-deficient rats for periods up to sixty-three days, R. E. Olson and N. O. Kaplan (*J. Biol. Chem.* **175,** *515 (1948)*) reported that the percentage of bound vitamin retained in liver, kidney, adrenal, and heart varied from 30 to 55 per cent of normal. Since these

values are somewhat lower than those obtained by the *Acetobacter* assay for rat tissues made deficient under comparable conditions, it is possible that PAC or related conjugates account for a larger portion of the total pantothenic acid in deficient tissue than in normal tissue.

This conclusion is substantiated by the results of dialysis of normal and deficient tissue by the Oregon group. By determining both the acetylating power (coenzyme A assay) and the *Acetobacter suboxydans* growth-stimulating power (total bound pantothenic acid including PAC) of extracts of kidney, liver muscle, and heart tissue from normal and pantothenic acid-deficient rats, before and after dialysis against running cold distilled water for forty-six to sixty-eight hours, it was possible to compare the relative amounts of coenzyme A and PAC in these tissues. Since coenzyme A is readily dialyzable, activity for *Acetobacter* remaining after dialysis is the bound pantothenic acid of PAC. These experiments indicated that from 20 to 37 per cent of the total bound pantothenic acid in normal tissues and from 24 to 57 per cent in deficient tissues were accounted for by PAC. Restriction of dietary glutamic acid had no effect upon the tissue levels of total bound pantothenic acid, suggesting that the endogenous synthesis of glutamic acid is not a limiting factor in the synthesis of pantothenic acid conjugates.

Regarding the structure of coenzyme A, F. Lipmann and his colleagues (*J. Biol. Chem.* **186,** *235* (*1950*)) have published their procedure for purification of coenzyme A from hog liver and proposed a tentative partial structure for the coenzyme. Their best amorphous preparation contained 11 per cent pantothenic acid, 20 per cent adenine, 22 per cent ribose, 9 per cent phosphorus, 12 per cent nitrogen, and 2 per cent sulfur. The sulfur was apparently present as cystine, and microbiologic analysis indicated that about 1 mole of glutamic acid per mole of pantothenic acid was present. This preparation assayed 130 units of acetylation activity per milligram of dry weight and was thought to be contaminated mainly by adenylic acid.

On the basis of these analyses and previous enzyme studies with diphosphoesterase and a liver peptidase (*Nutrition Reviews* **7,** *348* (*1949*)) Lipmann and co-workers postulated a tentative partial structure for coenzyme A which featured a pyrophosphate bridge between pantothenic acid and adenosine and a peptide linkage between pantothenic acid and glutamic acid. Support for this view is found in the experiments of G. D. Novelli, N. O. Kaplan, and F. Lipmann (*Fed. Proc.* **9,** *209* (*1950*)) who incubated coenzyme A with a dinucleotidase isolated from potato and found inactivation of the coenzyme. In the presence of adenosinetriphosphate (ATP) and a liver enzyme, the split products could be resynthesized to coenzyme A. This synthesis is analogous to those demonstrated by A. Kornberg (*J. Biol. Chem.* **182,** *779* (*1950*)) in which ATP was found to yield flavin-adenine-dinucleotide and diphosphopyridinenucleotide when added to flavinmononucleotide and nicotinamide-mononucleotide, respectively, in the presence of appropriate enzymes (*Nutrition Reviews* **8,** *48* (*1950*)).

T. E. King and F. M. Strong (*Science* **112,** *562* (*1950*)) have now reported the synthesis of three pantothenyl phosphates and they discuss certain of their biologic properties. Two monophosphates, one in which pantoic acid is esterified in the alpha-hydroxyl and one in which pantoic acid is esterified in the delta-hydroxyl, and the diphosphate were synthesized and tested for "free" pantothenic acid activity in the *Lactobacillus arabinosus* growth test, for "bound" pantothenic acid activity by both the *Acetobacter suboxydans* and the acetylation tests, and as precursors for coenzyme A in a crude pigeon liver system containing ATP. None of the esters showed any activity for either

the *Lactobacillus* or the *Acetobacter* before hydrolysis. All of these esters were alkali-stable, were difficultly split by myelase-P, papain, or pancreatin, and easily split by intestinal phosphatase. This latter mimics the behavior of both PAC and coenzyme A.

All of the phosphates were inactive in the acetylation test and only the diphosphate, apparently, was active in the crude liver system with ATP as a precursor for coenzyme A. These data suggest that the pantothenic acid in coenzyme A is esterified with phosphate at both hydroxyls of pantoic acid. It also raises the question of whether a glutamyl-*beta*-alanine peptide linkage is essential for the biologic activity of coenzyme A.

These fascinating studies on the nature of bound pantothenic acid and the structure of coenzyme A will no doubt be only a preface for the complete elucidation of the structure of both PAC and coenzyme A as well as the structural basis for the role of coenzyme A as an acetyl carrier.

FLUORIDE CONTENT OF HUMAN DIETS

Thirteen years ago, H. T. Dean (*Pub. Health Reports* **53,** *1443 (1938)*) reported on the role of fluorides occurring naturally in communal water supplies in the prevention of dental caries. It is, of course, well known that not only are fluorides at levels of 0.8 to 1.5 parts per million beneficial in decreasing the incidence of dental caries, but that amounts of fluorides in excess of these levels cause chronic, endemic dental fluorosis, "mottled enamel," and that prolonged ingestion by adults may result in hypercalcification of the spinal column with reduced mobility.

Apparently, drinking water is the most common and the major source of fluorides, and fluoride in the ratio of 1:1,000,000 in the water supply is considered enough for prevention of dental caries with a minimal occurrence of mottled enamel (H. T. Dean, "Fluorine and Dental Health," edited by F. R. Moulton, *Am. Assn. Advancement Sci., Washington, D. C. (1942)*). Some communities in Canada and the United States (*Nutrition Reviews* **3,** *115 (1945)*; **5,** *322 (1947)*) have even added fluorides to the drinking water in long-range experiments to evaluate the effects on caries and on potential pathologic manifestations.

Fluorides usually appear in foodstuffs in small amounts. From studies with experimental animals, soluble fluorides which occur in foods have been shown to be as effective as those in drinking water for prevention of tooth decay and for causation of toxic reactions. However, under ordinary situations of food selection by human beings, it has always been suspected, albeit without intensive investigation, that food sources of fluorides were sufficiently poor and infrequent as to contribute only insignificant amounts of fluorides. There is at least one known exception to this belief. The inhabitants on the lonely island in the South Atlantic Ocean, Tristan da Cunha, have a high incidence of the mild to moderate degrees of "mottled enamel" even though their drinking water supplies contained only 0.2 p.p.m. of fluorides, a level far below that reported necessary to cause mottling (R. F. Sognnaes, *J. Dent. Res.* **20,** *303 (1941)*). Analyses of these teeth indicated that they contained a higher fluoride content than teeth which developed in a nonfluoride region of this country (Sognnaes and W. D. Armstrong, *Ibid.* **20,** *315 (1941)*). In addition the dental caries incidence of the entire population of this island was much lower than would be expected in individuals with a low fluoride ingestion.

Studies of the food consumption of the islanders indicated that the main dietary com-

ponents were potatoes and fish accompanied by a rather high intake of tea. In cases of crop failure or in the "poorer" families who grew less potatoes than were required, potatoes were replaced to a large extent by fish. Three families whose fish consumption was measured over a period of a year consumed on the average 239, 383, and 392 g. of fish per individual per day. On the basis of an average fluoride content of 7.0 p.p.m., these individuals would each have consumed an average of 1.7, 2.7, and 2.7 mg., respectively, of fluorine per day or an equivalent of 1.7, 2.7, and 2.7 liters, respectively, of water containing 1 p.p.m. of fluorine per day. In addition to the amount supplied by fish, appreciable amounts of fluorides were contributed by the high potato consumption even though the fluoride content of potatoes is only in the neighborhood of 1.0 p.p.m., and also by the high intake of tea.

M. P. Ham and M. D. Smith (*Canad. J. Res.* (*Section F*) **28,** *227* (*1950*)) have undertaken studies to obtain information concerning the fluoride content of the diet common to people living in Southern Ontario, and some of the factors which influence it. Analyses were made of whole meals and beverages of various types. Meals were obtained from a local restaurant and prepared for sampling by mixing in a Waring Blendor.

In one series of analyses of Toronto meals, the food-borne fluoride appeared to amount to 0.18 to 0.30 mg. each day. Breakfast in this instance included fruit juice, cream of wheat, toast, and marmalade and was comparatively high in fluoride content. The dinner meal, consisting of tomato juice, halibut, spinach, bread, butter, and dessert, had the highest content chiefly because of the presence of fish and spinach, which generally are comparatively high in fluorides. These figures are somewhat lower than those reported by F. J. McClure (*Pub. Health Reports* **64,** *1061* (*1949*)).

Studies were also made which showed that tea infusion provided approximately 1 p.p.m. of fluoride which is the optimal content for water. Apparently cheap tea contains more fluorine than expensive tea. Cheap tea infusion contained 1.2 to 1.3 p.p.m. and expensive tea 0.9 to 1.1 p.p.m. It is suggested that this results from the fact that older leaves are used in cheaper tea and fluorine tends to accumulate with age (E. Reid, *Chinese J. Physiol.* **10,** *259* (*1936*)). A cola beverage contained 0.06 to 0.07 p.p.m. and the water in Toronto contained 0.11 to 0.12 p.p.m.; homogenized Toronto milk contained 0.06 p.p.m.

The authors wished to determine whether foods produced in low water-fluoride districts contained less fluoride than those produced in high water-fluoride areas and thus affected the total fluoride ingestion. Milk was the food selected for study of this relationship, because it is consumed by a large percentage of the population, and especially in large quantities by young children.

Some disagreement exists concerning the influence of fluoride present in the drinking water of cows on the fluoride contained in the milk produced. For this reason, two cities, Kitchener and Stratford, were chosen as suitable centers for the study. Figures obtained from the Ontario Department of Health showed that the water supply of Kitchener had an insignificant fluoride content while the Stratford water had the optimal fluoride content. The fluoride content of each city's water supply was determined for three consecutive months and homogenized milk from three dairies in each city was analyzed for fluorides for three consecutive weeks. The fluoride contained in the communal water supplies was found to be essentially that reported by the Department of Health.

The fluoride content of milk was rather low, ranging from 0.03 to 0.13 in Kitchener and in Stratford from 0.09 to 0.17. It was not felt that the differences were great enough to be of any particular significance. H. V. Smith, M. C. Smith, and M. G. Vavich

(*Univ. Arizona Agr. Exp. Sta. Bull.* **77,** (*1945*)) reported that even when cows are given sodium fluoride in high concentration in their drinking water (up to 500 p.p.m.), the fluoride was not transmitted in great quantities to the milk, but rather was excreted by the kidneys. Others frankly stated (E. B. Hart and C. A. Elvehjem, *Ann. Rev. Biochem.* **5,** *271* (*1936*); P. H. Phillips, Hart, and G. Bohstedt, *J. Biol. Chem.* **105,** *123* (*1934*)) that the fluoride content of milk is unaffected by ingestion of fluorides. However, J. F. McClendon, W. C. Foster, and G. C. Supplee (*Arch. Biochem.* **1,** *51* (*1942*)) believe that the fluoride content of milk varies directly in proportion to the fluoride concentration of the water consumed by the cows.

In the course of this study the authors also examined some common baby foods, because of the importance of child feeding. In two of these baby foods the fluoride content ranged from 14.3 to 13.7 p.p.m. It was assumed that most of this high content comes from the bone meal which constitutes about 2 per cent of one type of baby food. Bone meal capsules which were analyzed showed a content of 246 to 285 p.p.m. of fluoride content.

Apparently absorption of fluoride by the human subject depends on the actual solubility of the fluoride salt and its state at the time of ingestion (W. Machle and E. J. Largent, *J. Indust. Hyg. Toxicol.* **25,** *112* (*1943*); F. J. McClure, H. H. Mitchell, T. S. Hamilton, and C. A. Kinser, *Ibid.* **27,** *159* (*1945*)). The studies of the Canadian workers indicate that variations in the fluoride content of the diet as well as that of the water supply must be considered when an effort is made to determine the fluoride balance. However, their studies are but the barest introduction to the consideration of this problem in comparison to the need for further information. The attempt to evaluate effects of fluoride content of the water on the fluoride content of cows' milk needs to be extended to a wide variety of edible products. The need for this is particularly true in child feeding where the fluoride content of both the drinking water and the diet needs to be known.

ALLOXAN AND INSULIN SECRETION

Alloxan is a drug which has been used extensively to produce lesions of the pancreas conducive to diabetes. It is related chemically to such physiologically important substances as uric acid and riboflavin. While its mode of action in producing diabetes is not known with certainty, it has been generally assumed that it acted by stimulating to excess and consequently exhausting the islets of Langerhans (endocrine part of the pancreas) (F. D. W. Lukens, *Physiol. Rev.* **28,** *304* (*1948*)).

Claims that alloxan was normally present in animal tissues and fluids (R. S. Tipson and J. A. Ruben, *Arch. Biochem.* **8,** *1* (*1945*)) suggested the possibility that alloxan was a physiologic intermediary in the secretion of insulin. The likelihood of this mechanism was increased when it was demonstrated that the concentration of alloxan in the blood of rats was raised at least fiftyfold (to about 1 microgram per milliliter) twenty to forty minutes after administration of glucose (P. Schiøler, *Biochim. et biophys. acta* **2,** *260* (*1948*)).

That the damage to the pancreatic islets can also be caused by glucose itself, as demonstrated in the guinea pig by repeated intravenous infusions (C. A. Woerner, *Anat. Record* **71,** *33* (*1938*)) and in the rat by repeated intraperitoneal injections (F. C. Dohan and F. D. W. Lukens, *Endocrinology 42, 244* (*1948*)), could be interpreted on the basis of resulting exaggerated production of alloxan.

On the other hand, certain substances have

been found to inhibit the action of alloxan. One of the most effective of these substances is British anti-lewisite (BAL), which has been shown to block sulfhydryl (—SH) groups of organic molecules (A. Lazarow, *Proc. Soc. Exp. Biol. Med.* **61,** *441 (1946)*). Others include glutathione, cystine, and niacin (S. Banerjee, *Science* **106,** *128 (1947)*). If it could be proved that such substances inhibit insulin production in the normal animal while they had no effect in the diabetic animal, the probability that alloxan is a normal stimulant of insulin production would be increased. If, furthermore, even in the presence of insulin, the presence or functional integrity of the pancreas is necessary for this effect to be observable, then the possibility that these agents intervene at a different stage of carbohydrate metabolism from that of insulin secretion or that they act by inactivation of insulin, would be eliminated.

Experimental confirmation of these hypotheses was carried out by T. H. J. Huisman, W. Lammers, and P. Siderius (*Acta physiol. pharmacol. Neerland.* **1,** *184 (1950)*) and by Lammers, Siderius, and J. H. Gaarenstroom (*Ibid.* **1,** *193 (1950)*). It was shown that administration of BAL to normal rats increases the blood glucose. Simultaneous administration of glucose renders the hyperglycemic property of BAL even more manifest. No such effect is observed in eviscerated or alloxan-diabetic rats. Furthermore, the effect of BAL administration is not restored if the diabetic rats are treated with insulin during the experiment. Similarly, the second series of experiments, dealing with niacin, demonstrated that simultaneous administration of niacin and glucose to normal rats is followed by a higher level of hyperglycemia than that produced by administration of glucose alone. Again, this differential effect is dependent on the presence of the pancreas and is not seen when, instead of normal rats, eviscerated animals treated with insulin are used.

These data thus bring considerable circumstantial support to the idea that alloxan is a compound of normal physiologic significance and that it is an intermediary in the secretion of insulin. That, even if this theory is considered acceptable, seemingly contradictory evidence remains to be explained, is pointed out by Lammers and his collaborators when they recall that the time-lag between administration of alloxan and fall of blood glucose is often considerable, of the order of four to five hours (Lukens, *loc. cit.*), and that this hypoglycemia is preceded by hyperglycemia. This may in turn be due to a stimulation by alloxan not only of insulin secretion but of the production by the pancreas of H. De Waele's anti-insulin factor (*Nutrition Reviews* **8,** *319 (1950)*) as well.

Whatever the mechanism ultimately retained, the alloxan theory of insulin secretion appears to be a useful working hypothesis, both to the investigator studying the endocrine regulation of carbohydrate metabolism and to the clinician studying the etiology of hypoinsulinic diabetes.

FOOD ALLERGY

The extent to which food is important as an etiologic agent in allergic states has been the subject of much discussion. A major part of the difference of opinion arises from lack of agreement as to the reliability of the various methods which have been used in diagnosis. Skin testing, dietary history, intentional feeding, elimination diets, provocative diets, and rotary diversified diets have been used singly or in combination by various authorities in studying this subject, but no single method seems completely satisfactory. Management of these patients often becomes a matter of trial and error, a rather time-

consuming process. Some aspects of this problem have been reviewed (*Nutrition Reviews* **7,** *66 (1949)*).

H. Leibowitz, A. Chester, and H. Markow (*J. Am. Med. Assn.* **144,** *990 (1950)*) compared the results of skin testing with an intentional feeding procedure. Thirty-six adults and 34 children of both sexes were studied; 47 patients had chronic asthma and 23 had chronic allergic rhinitis. All patients were tested intracutaneously with fifty-six foods comprising eighteen fruits, eighteen vegetables, and twenty incidental foods.

Intentional feedings were started after completion of the skin tests and while the patients were undergoing specific hyposensitization therapy. The foods used in these tests were completely eliminated from the diet of the patient for one week and were then eaten in quantity three to four times a day prior to his return to the clinic. The patients were questioned as to whether the procedure caused any aggravation or return of symptoms which had previously cleared. Before these tests were considered to be positive, the test was repeated on two consecutive weeks. When only one of the three tests was positive, the result was recorded as negative. Intentional feeding tests were done not only to confirm the results of positive skin tests but also to discover clinical sensitivities where negative skin reactions were found. It is not clear whether the foods were fed singly or in groups.

All of the fifty-six foods tested intracutaneously caused reactions to some degree in some of the 70 patients, ranging from 9 positive reactions to peanut to 46 positive reactions to lettuce. Of 3920 skin tests performed, 44.2 per cent were positive. The foods most frequently causing positive skin reactions were lettuce, asparagus, spinach, corn, tea, sweet potato, cantaloupe, pea, peach, tomato, and corn meal. The commonly accepted belief that milk, wheat, and eggs are among the foods most frequently causing allergies was not confirmed according to these skin tests. Wheat was 34, milk 38, and egg 53 in the order of frequency of positive skin reactions. Fish (codfish) and nuts (almonds) were 55 and 56, while chocolate was 14 in the frequency list.

A very low incidence of reactions was found with the intentional feeding tests as compared with the high percentage of reactions with the skin tests. A total of 2467 feeding tests gave only 3.1 per cent positive reactions. Thirty-five of the fifty-six foods yielded one or more positive tests when given to individuals having positive skin reactions to the same food. Lettuce, which ranked first as a cause of skin reactions, gave but one positive feeding test.

Those foods giving the highest frequency of positive feeding tests in skin test-sensitive patients were chocolate 5, tomato 4, and pineapple 4, whereas they were 14, 10, and 27 in order of frequency of positive skin tests. Although 1732 skin tests were positive, confirmation could be obtained in only 52 (4.5 per cent) of the 1168 feeding tests which were done in these individuals. Milk, eggs, and wheat yielded only 3 positive feeding tests in 57 trials.

Among 1299 intentional feeding tests in negative skin test patients, there were 25 (1.9 per cent) positive reactions. The most frequent reactors in this group were peanut 3, almond 3, codfish 2, pepper 2, date 2, and chocolate 2. The first three of these foods were last on the frequency list for skin reactions.

The percentage of positive and negative skin tests was about the same for adults and children. The positive skin tests were confirmed by feeding tests in children about three times as often as in adults (6.9 per cent against 2.7 per cent). However, positive feeding tests with foods giving negative skin tests were noted twice as often in children as in adults (2.7 against 1.5 per cent).

When the over-all results were considered, 28 of the 70 patients showed positive intentional feeding tests to one or more foods. Fourteen of these patients gave positive feeding tests only with foods causing positive skin reactions, while 14 gave positive feeding

tests with positive and negative skin-reactive foods. Twenty-five of these 28 patients gave feeding tests and skin tests which were positive to the same food.

The authors conclude that the food skin test is reliable and important and that its use as a diagnostic tool should not be abandoned. They base this conclusion on the low (1.9 per cent) incidence of positive feeding tests in the face of negative skin tests, as compared with 4.5 per cent incidence of positive feeding tests in individuals with positive skin tests. While this conclusion may be perfectly correct, one cannot help but speculate whether a careful statistical analysis of the data would bear this out. Furthermore, 80 per cent of the positive skin tests and of the positive feeding tests showed only slight reactions. The differentiation of positive and negative feeding tests was apparently determined largely by information furnished by the patients who had already received the foods and who may have known to which foods they were skin sensitive. Such a method is certainly open to many errors.

The conclusion as to the value of the skin test is apparently also based on the assumption that the feeding test is the final proof of food sensitivity. There are those who would question this, especially when dealing mainly with subjective phenomena and 80 per cent slight reactions.

Demonstration of a food sensitivity in a given patient does not necessarily mean that this is the etiologic agent in an allergic condition. Perhaps the authors had this in mind when they concluded that foods play a minor role to pollens, inhalants, and bacteria in the majority of their cases, though by their own criteria 40 per cent of their patients were allergic to some food and showed some clinical exacerbation of their allergy when the food was consumed.

PHOTOCHEMICAL ACTIVITY OF RIBOFLAVIN

The vitamin riboflavin, in the form of flavoproteins, exercises an important function as an electron carrier in the oxidation of many metabolic products of carbohydrate and protein. Several other flavoproteins (e.g., xanthine oxidase, amino acid oxidase) are specific enzymes.

In addition to this type of function, however, there is mounting evidence that flavins may act as photoreceptor molecules and thereby induce or mediate other important metabolic reactions. A. W. Galston (*Science* **111,** *619 (1950)*) has briefly reviewed earlier connections between riboflavin and light, and has presented interesting experiments concerning plant growth and riboflavin.

For some time it has been known that riboflavin undergoes decomposition by light, giving lumichrome or lumiflavin according to the pH of the medium. Riboflavin is fluorescent, demonstrating its ability to absorb near-ultraviolet light and to emit visible light. Riboflavin has been reported of benefit in certain visual disorders, it is present in high concentration in the retina (of the codfish), and induces a photochemical oxidation of ascorbic acid.

Galston's preliminary experiments demonstrated that riboflavin in the medium used for plant tissue growth caused an increase in growth in the dark, but an inhibition resulted when the tissue was illuminated. The extent of inhibition depended on the level of the plant growth hormone indoleacetic acid in the medium, and the effect of riboflavin appeared to consist of a photochemically induced oxidation of indoleacetic acid.

Although it seems likely that the photooxidation of the hormone explains the above-recorded effect of riboflavin, the photochemical oxidation of other substances induced by riboflavin was also noted. Thus histidine particularly, but also tryptophan and methionine, were photochemically oxidized in

the presence of riboflavin. Furthermore, histidine linked in the dipeptide beta-alanyl histidine was likewise oxidized. The enzymes alpha-amylase, tyrosinase, and crystalline urease, and a bacteriophage tested, were also destroyed by light in the presence of riboflavin.

Galston recognized that most of the riboflavin in tissues is in a protein-bound form, hence the demonstration of a photochemical reaction involving flavoproteins might be of more physiologic significance. Such a photochemical activation of xanthine oxidase, a flavoprotein, had been shown earlier by F. Bernheim and M. Dixon (*Biochem. J.* **22,** *113 (1928)*).

Galston's first attempt to demonstrate light sensitivity of a flavoprotein enzyme was with D-amino acid oxidase, but no effect of light could be observed. Attention was then turned to a plant enzyme which occurs in etiolated pea epicotyls, an enzyme pertinent to the general study, since it oxidizes indoleacetic acid. This oxidase was found to be activated by light in its destruction of indoleacetic acid. By utilizing light of different wavelengths, it was found that the greatest activation of the enzyme occurred at the wavelength (440 mμ) characteristic of flavin absorption. The complete "action spectrum" plotted from activation at different wavelengths was characteristic of a flavin. It further seemed clear that a flavoprotein was the photoreceptor, since it was heat-labile and nondialyzable. Further details of the indoleacetic acid-inactivating system, which cannot properly be reviewed here, were presented by Galston (*loc. cit.*).

Evidence from the literature and further experiments were presented by the investigator to show that the photosensitivity of riboflavin in plants may be responsible for such phenomena as phototropism. It appears to be generally agreed that growth curvature caused by uneven exposure to light is caused by a difference in growth hormone distribution, and in the well-known *Avena coleoptile* the illuminated side has been found to contain a lower auxin content than the dark side.

Others had indicated that the natural growth hormone of the *Avena coleoptile* appears to be indoleacetic acid, and A. W. Galston and R. S. Baker (*Am. J. Bot.* **36,** *773 (1949)*) demonstrated that the rates of destruction of indoleacetic acid and the natural diffusible auxin from *Avena* were parallel. Thus it seemed probable that the natural growth hormone of *Avena* is indoleacetic acid and that riboflavin can act photochemically in the destruction of the hormone.

The role of riboflavin in plant photochemical reactions is somewhat contrary to previous interpretations since a carotenoid had been believed to be the photoreceptor substance. This was supported by the fact that blue light is most active for phototropism, and that beta-carotene is found in high concentration in photoreceptor parts of the plant. Actually riboflavin is also abundant in the *Avena coleoptile*. Unpublished experiments were mentioned to the effect that coleoptiles of a mutant strain of corn were devoid of carotenoids, although normal in flavin content, and showed a normal phototropic response.

Galston does not conclude that flavin pigment is the only photoreceptor in the phototropic response, but the demonstrated photosensitivity of certain reactions induced by riboflavin and flavoproteins should lead to much further experimentation on the role of this vitamin in the light-response reactions.

TOXEMIC-UREMIC SYNDROME IN PIGLETS

Dried skim milk diluted with water to approximate the composition of fresh cow's skim milk is not satisfactory as a diet for piglets (W. W. Green, H. C. H. Kernkamp,

M. H. Roepke, and L. M. Winters, *Am. J. Vet. Res.* **10,** *256 (1949)*). The original objective of the Minnesota investigators was to develop feeding practices for piglets that were separated from their dams at an early age, in the hope that diets could be evolved which would result in more vigorous and more rapidly growing pigs than is the case under ordinary conditions. However, a severe and often fatal metabolic disturbance was encountered when the piglets were fed diets that consisted chiefly of reconstituted cow's skim milk. The above-mentioned paper is devoted to a description of this nutritional disorder.

The disturbance displayed the characteristics of a toxemia that was manifested by nausea, vomiting, diarrhea, dehydration, itching, muscular jerks, stupor, and coma.

Piglets of inbred lines were used for these experiments. They were usually separated from their dams when approximately 7 days old. An interval of two or three days was allowed between the time of weaning and the experimental feeding. Whole cow's milk was fed during this period. The pigs were healthy and vigorous when the experiment began.

The basal diet was a fluid mixture of 15 per cent by weight of spray-process, dry, fat-free milk solids, and 85 per cent by weight of tap water. Supplements consisted of a mineral solution that supplied 0.10 mg. per pound of pig per day of each of the following components: cobalt sulfate, copper sulfate, and manganese sulfate; and 22.5 mg. per pound of pig per day of ferrous sulfate. Each pig also received daily 5 ml. of U.S.P. cod liver oil. Thirty-nine pigs were fed the basal diet; they ranged in age from 6 to 14 days when first placed on the diet. Of the 39 pigs, 36 either died or were killed when moribund, and all showed, in varying degrees, one or more of the clinical and pathologic characteristics of the toxemic syndrome.

Pigs that were 6 to 8 days old when started on the experimental diet usually became sick and died seven to eight days later, whereas 11- to 14-day old pigs survived somewhat longer (eleven to twelve days) on the experimental diet. In some cases the disease was acute and ran its course in three to four days, while in other cases it was chronic and extended over periods of ten days or more.

In addition to the symptoms mentioned earlier, the toxemic syndrome was associated with a rapid rise in urea nitrogen in the blood. The mean value of 10.5 mg. of urea nitrogen per 100 ml. of blood at the start of the experiment increased to 23.2 mg. per 100 ml. of blood after five days on the experimental diet. This rise continued so that by the thirty-second day of the experiment an average of 43.5 mg. of urea nitrogen per 100 ml. of blood was found in the survivors. The blood sugar values were 108.6 mg. per 100 ml. of blood initially and did not change greatly during the experiment (range 88.2 to 114.0).

The postmortem findings were indicative of toxemia. The kidneys were enlarged, pale, soft, and friable, and the capsule could be stripped off easily. Urates were often present in the renal pelvis. Microscopic changes in the kidneys consisted of granular and hydropic degeneration of the uriniferous tubules. Globules of fat were found in the epithelial cells of the distal end of the nephron and in the cells lining the pelvis. Other changes noted were fatty and degenerative changes in the liver, enlarged and pale adrenals, degenerative changes in the heart muscle, engorged mesenteric blood vessels, swollen edematous brains, and flaccid and toneless stomach walls.

Various diet modifications were tried in order to gain some information as to the cause of the toxemia. Increasing the energy value of the diet by including dextrin (15 per cent of dried skim milk, 11 per cent of dextrin, and 74 per cent of water) increased the severity of the toxemic-uremic syndrome. Two of 10 pigs that received this diet were dead within forty-eight hours and 5 more were prostrate or dead by the fifth day. On

the fifth day the 6 pigs in which symptoms were present had an average of 197.4 mg. of urea nitrogen per 100 ml. of blood.

A diet containing 15 per cent of dried skim milk, 3 per cent of Karo syrup, 82 cent of water, and fortified with brewers' yeast, was protective against the toxemia. The yeast was supplied initially at the rate of 5 g. per pig daily and was gradually increased to 12.5 g. per pig daily over a thirty-three day period. The pigs receiving this diet remained in an apparently healthy condition during the forty-five days of the experiment. Blood urea nitrogen values rose slightly during the first few weeks and then returned to normal.

Increasing the concentration of dried skim milk in the diet to 20 or 30 per cent increased the toxicity and produced an acute form of the disease. The pigs receiving the diet composed of 30 per cent of dried skim milk and 70 per cent of water were all dead in four days and the urea nitrogen was observed to increase from 18.0 to 87.1 mg. per 100 ml. of blood during the first twenty-four hours of the experiment.

The toxicity of the diet was reduced considerably when the skim milk concentration was reduced to 10 per cent. Inclusion of dextrin in the 10 per cent skim milk diet again aggravated the toxemia.

Yeast and cereal grass supplements at the rate of 1 pound per 100 pounds of diet were effective in reducing the toxic effects of a diet containing 10 per cent of dried skim milk, 8 per cent of dextrin, and 82 per cent of water.

In several experiments blood urea nitrogen determinations were made in pigs that were changed to protective diets after the appearance of symptoms of toxemia. The results indicated that diets containing yeast or cow's whole milk (3.5 per cent butterfat content) were helpful in correcting the high blood urea nitrogen associated with the toxemia.

The Minnesota investigators pointed out that the clinical, blood chemistry, and postmortem findings in the affected pigs simulated to a marked degree many of the findings which they have associated with a disease frequently seen in suckling pigs. No attempt was made to define the etiology of the apparent toxic nature of the skim milk diets. It was noted, however, that the syndrome may be related to the toxic manifestations that have been noted by several investigators when rats are fed skim milk or high concentration of lactose or galactose in diets low in fat (*Nutrition Reviews* **4,** *204* (*1946*); **6,** *185* (*1948*); **7,** *148* (*1949*); **8,** *104* (*1950*)).

The unusually high blood urea nitrogen values reported in the toxemic syndrome in pigs have, however, not been observed in the rat studies. P. Handler (*Nutrition Reviews* **6,** *185* (*1948*)) found moderate increases in the blood nonprotein nitrogen of rats receiving galactose diets but concluded that this abnormality could not be considered of primary importance. The syndrome in rats is accompanied by increased levels of total blood sugar and a marked increase in blood galactose, whereas in the studies with pigs hyperglycemia was not present. Yeast supplementation likewise does not improve lactose- or galactose-containing diets for rats, yet had an obvious protective action in the pigs receiving skim milk diets.

Despite these differences, it is of significance that cow's whole milk prevented the condition in pigs. Further studies no doubt will clarify whether the skim milk-induced disease in rats and the condition induced by the Minnesota workers in pigs are identical.

EFFECT OF SCURVY ON PHOSPHATASE ACTIVITY OF SERUM AND OF BONE

Alkaline phosphatase activity of serum has been observed to be decreased in infants or young children with scurvy as well as in scorbutic guinea pigs (*Nutrition Reviews* **1,** *136 (1943)*). However, it has not been definitively proved that this is the result of the specific deficiency, since other diseases which also are accompanied by weight loss or arrested growth produce a similar decrease in blood phosphatase (J. Smith, *Arch. Dis. Childhood* **8,** *215 (1933)*).

Results of experiments designed to assess the relative effects of scurvy and of the associated weight loss on the phosphatase content of serum and of bone have been reported by H. R. Perkins and S. S. Zilva of the Lister Institute in London (*Biochem. J.* **47,** *306 (1950)*).

Guinea pigs were the experimental animals used. The diet provided was either an ascorbic acid-deficient diet used in earlier experiments in the same laboratory or a stock-mixed diet composed of oats, bran, and cabbage. Animals were killed after selected intervals on a given diet and specimens of blood and bone taken for determination of phosphatase. The procedure included obtaining specimens of bone and cartilage from zones of active calcification in each animal. The material taken was that from costochondral junctions and from epiphyses of the proximal ends of the tibias. Phosphatase activity was determined by analysis of inorganic phosphate released after incubation of serum or aqueous extracts of the bone and cartilage in an alkaline medium containing a substrate of sodium beta-glycerophosphate. Enzyme activity was expressed in terms of milligrams of phosphorus liberated per hour per 100 ml. of serum or per gram weight of tissue.

In a first experiment young guinea pigs in the phase of rapid growth were maintained on the ascorbic acid-deficient diet for a period of twenty-one days. Observations related to changes in body weight and in phosphatase activity of serum and of bone. In these animals there was no change in the growth rate until the fifteenth day, when there occurred sudden cessation of growth and loss of body weight. Signs of scurvy occurred in a few animals as early as the eleventh day and these were marked in most animals by the time of growth inhibition.

Serum phosphatase was maintained at the initial level until the eleventh day when a sudden decrease began which was continuous through the last ten days of the experiment. The mean serum phosphatase in 12 animals decreased from an original value of 16 mg. to about 3 mg. of phosphorus per 100 ml. In contrast, significant reduction in the phosphatase content of costochondral junctions or epiphyses was delayed until the fifteenth day, at which time a striking decrease occurred which paralleled the loss in body weight. Final activity of the enzyme in these bony structures was from one-third to one-half of the initial level. These findings were representative of the changes of phosphatase in serum and in bone found during the development of scurvy in other experiments.

The influence of weight loss unassociated with ascorbic acid deficiency on serum and bone phosphatase was then determined. Young guinea pigs were fed the scorbutic diet plus 25 mg. of ascorbic acid daily but with the diet so restricted in quantity that there was a loss of 20 per cent in body weight in nine days. Under these conditions loss of body weight began immediately and was paralleled by reduction in phosphatase content of serum and bone. After nine days on the restricted diet the percentage decrease in enzyme activity was approximately the same in serum, costochondral junctions, and epiphyses, about 50 per cent of initial values. These animals were then provided the stock diet ad libitum. Rapid growth was immediately restored and within six days the

weight lost during the nine days of restricted food intake was regained. Phosphatase activity of serum and bone responded promptly, increasing within twelve days to original levels.

In another experiment the scorbutic diet was administered in similarly restricted quantity for nine days but without the supplement of ascorbic acid. When the stock diet was given for an additional period of twelve days after food restriction, growth was resumed at a relatively slower rate and the weight lost during the earlier phase of the experiment was not fully regained. A second period of weight loss intervened after nine days on the stock diet and signs of scurvy appeared. There was reduction of serum and bone phosphatase during the period of restricted diet similar to that observed in the earlier experiment when a supplement of ascorbic acid was administered. However, in this instance there was no tendency for the phosphatase content of these tissues to increase when the stock diet was provided and the serum phosphatase decreased further several days prior to the onset of scurvy, while a less marked reduction in bone phosphatase occurred at the time of weight loss associated with scurvy.

The role of scurvy was eliminated in another experiment by feeding the scorbutic diet plus a daily supplement of 25 mg. of ascorbic acid, this diet being administered in such daily quantities that the body weights of the animals were controlled to reproduce the change of weight observed when young guinea pigs were fed the scorbutic diet in restricted amounts for nine days and then the basal diet for an additional period of twelve days. The decrease in concentration of serum and bone phosphatase occurred as it had before during the period of restricted feeding but remained at these levels during ingestion of the stock diet. Therefore, it was concluded that the secondary reduction in bone and serum phosphatase observed in the earlier experiment was in reality the result of scurvy and not of weight loss.

These experiments were repeated in adult guinea pigs in which processes of ossification were proceeding at a much slower rate. After twenty-eight days on the ascorbic acid-deficient diet evidences of scurvy occurred in the adult animals. During this period there was a significant reduction in the phosphatase of serum but not in that of bone. In addition, there was no change in the phosphatase content of these tissues when adult guinea pigs were provided the scorbutic diet plus a supplement of ascorbic acid, the quantity of diet being restricted to effect a loss of 20 per cent of body weight in twenty-five days.

The authors demonstrated that daily administration of 2 mg. of ascorbic acid would prevent scurvy in young guinea pigs fed the scorbutic diet. This quantity of ascorbic acid given as a daily supplement also protected against reduction in serum and bone phosphatase. Much larger quantities of the vitamin were required to effect tissue saturation. The phosphatase content of bone and serum cannot, therefore, be assumed to be dependent on high concentrations of ascorbic acid in tissues.

Ascorbic acid was also effectively demonstrated to correct the lowered phosphatase activity of serum and bone in guinea pigs with scurvy. Within two days of the beginning of administration of 25 mg. of ascorbic acid orally per day to guinea pigs which had been on the scorbutic diet for eighteen to twenty-one days, there was a marked increase in the phosphatase content of serum and of bone. By the fourth day concentrations of the enzyme in these tissues were well within normal ranges. It is of interest that these changes occurred before resumption of growth and gain in weight. It is unlikely that ascorbic acid directly regulates or determines phosphatase content, since no rise in phosphatase concentration of serum or bone was determined eighteen hours after parenteral injection of 25 mg. of the vitamin.

Perkins and Zilva (*loc. cit.*) have provided evidence that reduction of the phosphatase

activity in serum and bone occurs as the result of ascorbic acid deficiency and that this effect may be differentiated from that due to cessation of growth or weight loss. Information which is presently available does not permit conclusions concerning the significance of this finding in the animal economy. Whether the reduction of phosphatase in these tissues results from interference in synthesis of the enzyme or from mobilization for utilization in tissues affected by the deficiency cannot be stated.

NUTRITION IN LIVER DISEASE

The relation of faulty food intake to the genesis of liver disease has been a fascinating and much investigated problem in recent years. Soon after the discovery of insulin it became apparent that pancreatectomized dogs maintained with this hormone developed fatty livers which could be prevented or cured by administration of methionine or choline. This lipotropic action of methionine is not present when these dogs are fed whole protein even if it contains adequate methionine. If, however, the protein is hydrolyzed, lipotropic action is evident. Some investigators have concluded that the deficiency is of the external secretion of the pancreas, especially of the proteolytic enzymes.

Rats and other animals maintained on diets deficient in protein, particularly in the sulfur-containing amino acids, also develop fatty livers which may be prevented by administration of choline or methionine. No pancreatic disease is known to be present in this instance. Cirrhosis of the liver in chronic alcoholics is thought to be caused, at least in part, by a drastic reduction in the protein (methionine and choline) intake by the individual.

Others of the human species, notably natives in Africa, and perhaps individuals in many other tropical areas of the world, develop fatty livers while consuming food high in carbohydrate and low in fats and in proteins (especially those of animal origin). This condition which has been so amply described by J. Gillman and T. Gillman (*Nutrition Reviews* **5,** *353 (1947)*) and by J. N. P. Davies and H. C. Trowell (*Ibid.* **6,** *270 (1948)*) at first might be thought to be related directly to dietary deficiencies of choline or methionine, or both. This does not seem to be the case. Moreover, more recent evidence has indicated that although these patients do not have steatorrhea under ordinary circumstances, when they are brought to the hospital and fed an adequate fat diet, steatorrhea often does occur. Finally, extensive fibrosis and sometimes cystic degeneration of the pancreas is usually if not regularly observed in these patients. The cause and effect relation of this pancreatic disease has not been made entirely clear.

G. E. M. Scott (*Med. J. Australia* **1,** *37 (1950)*) has added to the knowledge of the problem by discussion of celiac disease, the celiac syndrome, pancreatic fibrosis, and its relation to nutritional deficiency and fatty liver disease. S. Farber (*Arch. Path.* **37,** *238 (1944)*) in a discussion of pancreatic function and pancreatic disease in early childhood noted the occurrence of a disease characterized by cystic fibrosis of the pancreas and fatty infiltration of the liver. Bronchiectasis was also a prominent finding.

Scott (*loc. cit.*) studied 16 patients at the Children's Hospital in Melbourne, each with a diagnosis at autopsy of fibrocystic disease of the pancreas. Commenting upon Farber's interpretation of the syndrome as a congenital or familial disease, Scott believes that at least in some instances a "toxic" or infectious factor may be of importance etiologically. In fact, the pancreatic lesions which he saw were essentially those of chronic pancreatitis, while the epithelial metaplasia in the pancreatic ducts was

thought to be due to vitamin A deficiency and the cystic change to loss of acinar tissue.

Finally, in addition to "toxic" and infectious factors, malnutrition and vitamin deficiency were considered by Scott to be highly important in the genesis of the pancreatitis. Pathologic disturbances of the gastrointestinal tract were usually found in the patients and were thought to be the direct result of nutritional deficiency, influencing secondarily the maintenance of normal pancreatic function and integrity. Thus, infection and dietetic factors were thought to be primary, leading to gastroenteritis with greenish, offensive stools. Pancreatic disease was thought to occur as a result, with steatorrhea as an early sign. Malnutrition of a more severe grade would then set in with evidence of specific nutritional deficiencies such as an enlarged fatty liver and epithelial metaplasia with loss of ciliated epithelium and perhaps even rickets.

Although this concept of the etiology of pancreatic fibrosis is not entirely supported by fact, it is stimulating and points again to the possibility that the pancreas may respond to malnutrition by hypofunction and pathologic degeneration and fibrosis leading secondarily to further malnutrition and to liver disease. Consideration of this matter with respect to genesis of the fatty liver disease seen in the tropics seems important. Perhaps the deficient diet together with infection lead first to pancreatic disease, with liver disease secondary to defective digestion and absorption of essential nutrients, especially methionine.

INTRAVENOUS IRON

Intravenous administration of iron preparations in therapeutic amounts has been almost impossible because of alarming reactions. Many different types of iron compounds have been tried and, in each instance, abandoned. However, sufficient work has been done with these preparations to demonstrate, in patients with hypochromic anemia, that injected iron is converted almost quantitatively into hemoglobin, that reticulocytosis is rapid, and hemoglobin regeneration maximal (*Nutrition Reviews* **5,** *150 (1947)*; **8,** *76 (1950)*). Thus a safe, effective form of iron for parenteral use would have some importance.

An iron-sucrose preparation (saccharated iron oxide) has been used for several years by British investigators in the treatment of iron deficiency anemia. The therapeutic response has been good and reactions have been infrequent and mild. This type of preparation has apparently been used infrequently in this country. E. B. Brown, C. V. Moore, C. Reynafarje, and D. E. Smith (*J. Am. Med. Assn.* **144,** *1084 (1950)*) have described their experience with this type of compound.

Three different preparations of saccharated iron oxide from three different manufacturers were used. The material is apparently a negatively charged colloidal solution of ferric hydroxide, stabilized by adsorption of alkali and sugar. The preparations contain 100 mg. of iron per 5 ml., though the exact composition and exact methods of manufacture have not been announced. No differences were noted in the safety and effectiveness of the three preparations.

Saccharated iron oxide was given intravenously to 10 patients with hypochromic microcytic anemia. The primary cause of iron deficiency in each instance was hemorrhage, either from the uterus or from the gastrointestinal tract; contributory causes in several patients were undoubtedly hypochlorhydria and poor diet.

Initial hematologic values showed the following range of variation: red blood cells

3.14 to 5.17 million, hemoglobin 5.8 to 9.8 g., and mean corpuscular hemoglobin concentration 25 to 31 per cent. Some patients received 40 mg. of iron the first day, 60 mg. the second day, and 100 mg. daily thereafter. In a few instances the initial dose was 100 mg. A few patients were given as much as 200 mg. of iron twice within a twenty-four-hour period. The total dosage of 700 to 2000 mg. was regulated in each patient by the amount of iron necessary to correct the calculated iron deficit.

All patients showed excellent clinical and hematologic responses; appetites improved and weakness became less pronounced. A reticulocyte peak occurred in all cases within six to eleven days, being greater than 15 per cent in all but 3 patients, and reaching a level of 21.2 per cent in one case. The average daily rise in hemoglobin varied from 1.106 to 0.262 g. per 100 ml., averaging 0.177 g.

Reactions were minimal. Venous thromboses at the site of injection did not occur. One patient complained of nausea, abdominal cramps, and a chill after receiving the first dose of 100 mg. of the iron compound. Subsequently the dose was reduced to 40 mg. and then gradually increased to the original 100 mg. level without further reaction. Another patient reported transient pain along the course of the injection vein. In one patient several milliliters of the saccharated iron oxide were accidentally infiltrated round the injection vein causing an acute local reaction, from which the acute discomfort subsided after ninety-six hours. Induration persisted for several weeks.

More important perhaps than the minimal reactions described above is the problem of overdosage. With oral iron administration the body, under most circumstances, has the capacity to regulate the amount of iron absorbed according to the amount needed. Rather large amounts of iron can be given orally over a period of years without excessive accumulation in the tissues or other evidence of harm. However, very little iron is excreted, except by hemorrhage, once the iron reaches the tissues. Thus it is possible that prolonged administration of intravenous iron might lead to overdosage, accumulation in the tissues, and perhaps result in conditions comparable to hemochromatosis, transfusion hemosiderosis, and similar conditions. From the practical standpoint this might pose a serious objection against the use of any intravenous iron preparation.

The St. Louis investigators review the extensive clinical and experimental literature on conditions characterized by excessive iron deposition in the tissues and from this information, and from their own experience, they conclude that although it is unquestioned that in hemochromatosis, transfusion hemosiderosis, and cytosiderosis excessive amounts of iron are associated with pathologic changes in several organs, it is not certain that the iron is actually responsible for precipitating these abnormalities.

Brown, Moore, Reynafarje, and Smith (*loc. cit.*) performed an experiment on 4 dogs giving saccharated iron oxide intravenously at two dosage levels (0.5 and 1.0 g. of iron per kilogram of body weight). The amount of iron injected daily varied from 100 to 300 mg.; the time required to inject the total dosages of 4.54 to 9.44 g. varied from forty-four to sixty-eight days. These dosages were selected to approximate in the dog the amount of iron usually found in the tissues of patients with hemochromatosis.

The dogs were fed a diet of commercial dog chow and were observed for fifteen months. They continued to be active and healthy in appearance; their weight was constant or increased slightly; and hematologic values remained normal. No abnormalities of liver function were detected by several tests. Fasting blood sugars were normal; serum iron levels were high during the period of injection but returned to a high normal after the iron had been given.

Biopsy specimens were taken from several

organs and tissues before and six months after the iron treatment. The control sections showed no abnormalities. The specimens taken six months later showed extensive deposition of iron-containing substances in many of the organs, but despite this there were no secondary changes such as fibrosis and cirrhosis as seen in hemochromatosis. There was nothing from the physiologic or anatomic viewpoint to indicate dysfunction of these organs despite the extensive iron deposits in the tissues. The authors emphasize, however, that their period of observation has been short and abnormalities may yet develop. Until this situation has been clarified they strongly recommend withholding excessive amounts of intravenous iron from human subjects.

Actually there are few real indications for intravenous administration of iron. This study does not show nor does it purport to show that intravenous iron therapy is any more effective than adequate oral therapy would have been in the usual patient with simple hypochromic anemia. However, 2 patients had been unable to tolerate oral iron but could tolerate and responded to the intravenous iron.

The following situations are ones in which intravenous iron should be helpful: (1) Hypochromic anemia in patients with ulcerative colitis, with functioning colostomy, after extensive bowel resection, and with sensitivity to iron. (2) Hypochromic anemia in patients refractory to oral therapy. Such patients may not respond to intravenous therapy either, but it might be tried. (3) It is also possible that intravenous iron might be helpful in hypochromic pregnant women seen for the first time just a few days before delivery. Enough of the iron might pass the placental barrier to give the infant some protection against anemia.

The authors give two simple formulas which can be used to calculate the amount of intravenous iron which should be given to any patient. The aim should be to give only enough to replace the iron deficit. Any excess may well be harmful. Should this preparation come into common use, the possibility of overdosage would have to be carefully watched.

NOTES

Letter to the Editor

Dear Sir:

I read with a great deal of interest the review of my article, in the September 1950 issue of *Nutrition Reviews*, under the title "Diet Therapy in Psychoneurotic Patients."

I was very much interested in your comments and I think the criticisms which you might make are perfectly justifiable. I am sure that one who reads an article of that type would have many questions which would come to his attention. Of these 772 patients, of course they were patients who came to me because they had psychogenic disturbances of the gastrointestinal tract, many did have fatigue. This fatigue, however, did not show the usual periodicity as seen in patients who had a so-called "flat curve." By this I mean it was not regularly present in the morning; may or may not be present before noon, and may or may not be most severe towards the end of the afternoon.

Secondly, I was not bringing any new concept of what hyperinsulinism might do to the individual. What I really tried to emphasize was that there could be a definite dietary and pharmacologic approach. I am convinced, from the evidence I have seen in ambulatory patients and from the patients who have been subjected to vagotomy or vagectomy for the surgical treatment of their peptic ulcer, that the vagus nerve is definitely at fault, or that it is the most logical pathway for emotional disturbances to travel from the brain to the pancreas.

I have, routinely, done intravenous glucose tolerance tests before and after vagotomy. In every patient the postvagotomy test has higher values than the preoperative tests, irrespective of whether the patient had a so-called "flat curve" or one slightly lower than normal. This, to me, is rather significant since I have observed some of these patients over a period of three and a half to four years and have had an opportunity to repeat their tolerance curves.

This raises a very serious question in my mind. If in our attempt to produce a more lasting cure for peptic ulcer with a vagotomy, may we not at the same time produce a potential diabetic? The problem, of course centers around the possible atrophy of the islet cells. Even though my indications for vagotomy are hard and fast, and not a routine procedure, I still am somewhat concerned because of the results of these glucose tolerance studies. Again I want to thank you for your very careful and critical review of my most recent article.

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Nutrition in the Public Schools

During the past several years a research study in nutrition education in the public schools has been conducted by the Department of Nutrition of the Harvard School of Public Health, Boston. The study was started in the public schools of Newton, Massachusetts, and now is concentrated in the public schools of Rutherford County, Tennessee, and Ascension Parish, Louisiana. The purposes of the study were to determine: (1) whether nutrition education could be incorporated into the ordinary curriculum of the public schools as a part of the health education, science, social studies, reading, arithmetic, and other studies, and (2) whether this nutrition education would in-

fluence the food habits, attitudes, and knowledges of children so that their daily intake of basic foods and understanding of the importance of food in health would be improved.

We recognize that nutrition education is more readily adaptable to the curriculums of the elementary grades; however, at the junior high and high school levels, there is already much in the specific curriculums of health education, science, social studies, home and family living, and other related fields, where nutrition and kindred subject matter are being taught. When nutrition education is definitely included in the elementary grades, and then re-emphasized in the junior high and high school grades, attainment of the "Goals for Nutrition Education" can be accomplished.

The "Goals for Nutrition Education" were formulated as the initial part of this study to serve as a guide to indicate the subject matter that should be developed to improve desirable habits, attitudes, and knowledges concerning food, nutrition, and health. The "Goals" are progressive and are divided into those for the early elementary (kindergarten —grade 3), later elementary (grades 4–6) junior high (grades 7–9), and senior high school levels.

The second publication of this research study on nutrition education in the public schools was the booklet "Activities—in Nutrition Education for Kindergarten Through Sixth Grade." It relates some of the school activities and illustrates some of the methods and technics by which teachers may incorporate nutrition education into their daily classrooms. These activities are listed by grades to facilitate use of the booklet; however, they may be readily employed at other grade levels by adaptation to the children's needs and abilities.

The third publication of this study has just appeared. It is entitled "Activities—in Nutrition Education, a Unit for High School Classes." It offers teacher and student information, suggestions for activities for classroom and laboratory, directions for conducting a three-day dietary survey of students, and a classroom test for knowledge. It has been prepared by a biology teacher, Dr. Elizabeth A. Lockwood, for the purpose of helping biology teachers to include a nutrition unit in their regular courses. This is not to imply that the unit is for biology teachers only. It is believed that much of the material included here may be helpful to all teachers who are concerned with guiding high school students to form good food habits and gain a better understanding of nutrition. The units may serve as a resource unit for courses in health, home economics, general science, hygiene, and other appropriate areas of the curriculum. It is offered as an example of how nutrition information and nutrition education can be included as a regular part of the high school curriculum.

All three of these educational booklets are available from The Nutrition Foundation, Chrysler Building, New York City, at cost, the two "Activities" booklets at $1.00 each and the "Goals" at $0.25.

Recent Nutrition Books

Biochemistry of Glucuronic Acid. Neal E. Artz and Elizabeth M. Osman. Academic Press, Inc., New York, 1950. Pp. 103. Price: $2.50.

Sanitary Milk and Ice Cream Legislation in the United States. (A study of laws and ordinances establishing sanitary standards for milk, cream, and ice cream.) Prepared in collaboration with the Committee on Milk Production, Distribution, and Quality. A. C. Dahlberg and H. S. Adams. National Research Council Bulletin No. 121, Washington, D. C. July 1950. Pp. 59.

Some Aspects of Food Refrigeration and Freezing. FAO Agricultural Studies No. 12. Washington D. C. November 1950. Pp. 205. Price: $2.00.